



DNA barcoding of Dutch birds

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Abstract

The mitochondrial cytochrome *c* oxidase subunit I (COI) can serve as a fast and accurate marker for the identification of animal species, and has been applied in a number of studies on birds. We here sequenced the COI gene for 387 individuals of 147 species of birds from the Netherlands, with 83 species being represented by > 2 sequences. The Netherlands occupies a small geographic area and 95% of all samples were collected within a 50 km radius from one another. The intraspecific divergences averaged 0.29% among this assemblage, but most values were lower; the interspecific divergences averaged 9.54%. In all, 95% of species were represented by a unique barcode, with 6 species of gulls and skua (*Larus* and *Stercorarius*) having at least one shared barcode. This is best explained by these species representing recent radiations with ongoing hybridization. In contrast, one species, the Lesser Whitethroat *Sylvia curruca* showed deep divergences, averaging 5.76% and up to 8.68% between individuals. These possibly represent two distinct taxa, *S. curruca* and *S. blythi*, both clearly separated in a haplotype network analysis. Our study adds to a growing body of DNA barcodes that have become available for birds, and shows that a DNA barcoding approach enables to identify known Dutch bird species with a very high resolution. In addition some species were flagged up for further detailed taxonomic investigation, illustrating that even in ornithologically well-known areas such as the Netherlands, more is to be learned about the birds that are present.

Keywords

Aves, conservation, cytochrome c oxidase subunit I, COI, taxonomy

Introduction

DNA barcoding is used as an effective tool for both the identification of known species and the discovery of new ones (Hebert et al. 2003, 2010, Savolainen et al. 2005). The core idea of DNA barcoding is based on the fact that just a small portion of a single gene, comprising a 650 to 700 bp fragment from the first half of the mitochondrial cytochrome c oxidase subunit I gene (COI), shows a lower intraspecific than interspecific variation. An attribute which characterizes a threshold of variation for each taxonomic group, above which a group of individuals does not belong to the same species but instead forms an intraspecific taxon. In other words, the recognition of patterns in sequence diversity of a small fragment from the mtDNA genome has led to an alternative approach for species identification across phyla.

Initially, DNA barcodes were proposed for the Animal Kingdom in 2003, when Hebert and colleagues tested a single gene barcode to identify species and coined the term 'DNA barcoding' (Hebert et al. 2003). Since that time COI sequences have been used as identifiers in the majority of animal phyla including vertebrates (e.g. Hebert et al. 2004, Ward et al. 2005, Kerr et al. 2007, Smith et al. 2008, Nijman and Aliabadian 2010, Luo et al. 2011) and invertebrates (Hajibabaei et al. 2006, Bucklin et al. 2011, Hausmann et al. 2011). In recent years, the practical utility of DNA barcodes proved to be an appealing tool to help resolve taxonomic ambiguity (Hebert et al. 2004, 2010), to screen biodiversity (e.g. Plaisance et al. 2009, Naro-Maciel et al. 2009, Grant et al. 2011), and to support applications in conservation biology (Neigel et al. 2007, Rubinoff 2006, Dalton and Kotze 2011).

Birds are among the best-known classes of animals and thus provide a taxonomically good model for analyzing the applicability of DNA barcoding. In the last seven years some 30 scientific papers have been published on the DNA barcoding of bird species, which combined have been cited 500 times (V. Nijman, unpubl. data April 2013). Most of the studies have shown that from this small fragment of DNA, individuals have been identified down to species level for 94% of the species in Scandinavian birds (Johnsen et al. 2010), 96% in Nearctic birds (Kerr et al. 2009a), 98% in Holarctic birds (Aliabadian et al. 2009) and 99% in Argentinean and South Korean birds (Kerr et al. 2009a, Yoo et al. 2006). Species delineation relying on the use of theshold set to differentiate between intraspecific variation and interspecific divergence has been criticized as leading to too unacceptable high error rates especially in incompletely samples groups (Meyer and Paulay 2005). However, even the critics of DNA barcoding concede that DNA barcoding holds promise for identification in taxonomically well-understood and thoroughly sampled clades. Birds are taxonomically well-known, especially those of the Western Palearctic to which the Netherlands, our study area, forms part. As noted by Taylor and Harris (2012), compared to other taxa that have been subjected to DNA barcoding, DNA barcoding studies of birds tend to represent aggregations of very large number of bird species barcodes. These often include (near) cosmopolitan species with samples from distant geographic locations potentially increasing the amount of interspecific variation in COI.

Here we explore the efficiency of identifying species using DNA barcoding from a large set of sympatric bird species in the Netherlands. Compared to previous studies on birds, our study area covers a very small geographic area, allowing to directly test the functionality of DNA barcoding 'in one's backyard'.

Methods

Sampling

The Netherlands is a small, densely populated country in northwestern Europe, with a land surface area of some 34,000 km², and ornithologically it is arguable one of the best-covered countries (Sovon 2002). The tissue samples used for sequencing were collected from breeding areas in the Netherlands, excluding oversees dependencies. Given the small size of the country some 95% of the samples were collected within a 50 km-radius of each other. Samples were part of the tissue collection of the Zoological Museum of Amsterdam (ZMA), which were recently relocated and deposited in the Naturalis Biodiversity Center, Leiden. Most were collected in the period 2000-2012 by a network of volunteers, ringers, airport staff, and bird asylums; no birds were specifically collected or killed to be included in the collection of the ZMA. Species and subspecies identification was based on morphology and when necessary, external measurements. These identifications were done by authors HvB and CSR, with the help of Tineke G. Prins. Individual birds were frozen upon arrival to be thawed and skinned at a later date, and indeed many birds arrived frozen. Samples were mostly taken from the bird's pectorial muscles, because of its size and easy access, and stored in 96% ethanol. Species nomenclature follows the taxonomy of Dickinson (2003). The complete list of sampled specimens including information about vouchers and trace files is available from the project 'Aves of the Netherlands' at the BOLD website (http://www.barcodinglife.com/).

PCR and sequencing

The tissue samples were subsampled and subjected to DNA extraction using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. PCR and sequencing reactions were performed, mainly following the same protocols described in Förschler et al. (2010), but with some minor modifications. Polymerase chain reaction (PCR) amplifications were initially performed using standard primers BirdF1 (TTCTCCAACCACAAAGACATTGGCAC) and BirdR1 (ACGTGGGAGATAATTCCAAATCCTG). When amplification was unsuccessful, alternate reverse primer BirdR2 (ACTACATGTGAGATGATTCCGAATCCAG) was used in combination with BirdF1 or alternate primer pair CO1-ExtF (ACGCTTTAACACTCAGCCATCTTACC) and CO1-ExtR (AACCAGCATATGAGGGTTCGATTCCT) was used (Hebert et al. 2004, Johnsen et al. 2010).

All PCRs were run under the following thermal cycle program: 3 min at 94 °C followed by 40 cycles of 15 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and a final elongation of 5 min at 72 °C. For each reaction the PCR mixture consisted of 2.5 μ l Qiagen Coral Load 10 × PCR buffer, 1.0 μ l of each 10mM primer, 0.5 μ l 2.5 mM dNTPs, 0.25 μ l 5U/ μ l QiagenTaq DNA polymerase, 18.75 μ l milliQ and 1.0 μ l template DNA for a total volume of 25 μ l. Bi-directional sequencing was performed for all specimens at Macrogen. We checked the possible amplification of pseudogenes (Numts) by translating the protein coding genes into amino acids sequences, but we did not observe any unexpected stop codons, frameshifts or unusual amino acidic substitutions. Furthermore we amplified a longer sequence of the COI gene with primers (CO1-ExtF and CO1-ExtR) for selected samples, and also here we did not see any indication of pseudogene co-amplification. Lijtmaer et al. (2012) found that, in birds, full-length COI pseudogenes are uncommon noting that they might be more frequently encountered when working with avian blood samples as opposed to muscle tissue samples (as used in here).

Data analysis

Sequences shorter than 500 bp and containing more than 10 ambiguous nucleotides were excluded from the analyses. All sequences have been deposited in GenBank (Accession numbers KF946551 to KF946937). A full list of the museum vouchers, for all specimens applied in this study, is provided in Appendix – Table 1.

For all sequence comparisons, the Kimura 2-parameter (K2P) model was used, because it is shown to be the best metric to compare closely related taxa (Nei and Kumar 2000, but for a contrasting view see Srivathsan and Meier 2012). Average intraspecific distances were calculated for those species that were represented by at least two specimens using Mega v5.1 software (Tamura et al. 2011).

For a group of birds that expressed a larger than expected intraspecific variation, the Sylvia warblers, we created a phylogenetic tree and created a haplotype network. We chose GTR+G+I as the best-fitting model of nucleotide substitution based on its Akaike's information criterion as implemented in JModelTest v0.1.1 (Posada 2008). A maximum likelihood (ML) tree was constructed in PAUP* v4.0b10 (Swofford 2002) using a heuristic search with the tree-bisection-reconnection branch-swapping algorithm and random addition of taxa. Relative branch support was evaluated with 500 bootstrap replicates (Felsenstein 1985). A minimum spanning haplotype network was constructed using a statistical parsimony network construction approach as implemented in TCS software package (Clement et al. 2000). This programme calculates the number of mutational steps by which pairwise haplotypes differ and computes the probability of parsimony (Templeton et al. 1992) for pairwise differences until the probability exceeds 0.95. The number of mutational differences associated with the probability just before the 0.95 cut-off point is then the maximum number of mutational connections between pairs of sequences justified by the parsimony criterion; these justified connections are applied in the haplotype network (Clement et al. 2000).

Results

A total of 387 sequences for 141 species (representing at least 158 taxa) were retrieved, including 52% of the breeding bird species in the Netherlands (Supplementary Table 1). The average number of sequences per species was 3.36 (range 1-13), with 83 species (59%) represented by more than two sequences. The mean K2P-divergence within species bears no significant relationship with sample sizes, i.e. number of sequences per species ($R^2 = 0.001$, p = 0.465). The mean intraspecific K2P-distance was 0.29% (range 0-8.68%) some 30 times lower than the mean intrageneric K2P-distances (9.54%, range 0-27.71%) (Table 1, Figure 1).

In general, 95% of species (134 species) showed a unique DNA barcode (these included the 58 species for which we only sequenced single individuals), while six congeneric species shared the same barcode and the mean intraspecific distance of them fell well below the threshold of species based on distance-based criterion (Hebert et al. (2003) 10 x rule). These congeneric species mostly included circumpolar species with close morphological similarities (Table 2).

Table 1. Comparisons of K2P-pairwise distances within various taxonomic levels for 83 species of birds from the Netherlands for which two or more sequences were available. Distances are expressed in percentages.

	Individuals	Taxa	Comparisons	Distances		
				Minimum	Mean ± S.E.M.	Maximum
Within Species	340	83	805	0	0.294±0.001	8.683
Within Genera	203	23	794	0	9.544±0.004	15.849
Within Families	282	20	2519	5.809	14.467±0.001	20.473

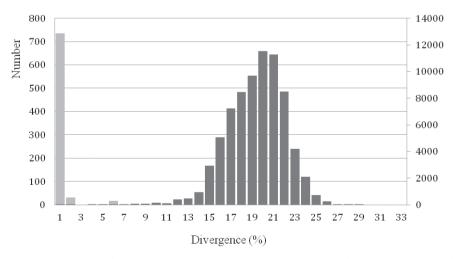


Figure 1. Comparisons of K2P-pairwise distances based on the COI gene of 141 species of birds from the Netherlands, showing a clear barcoding gap. Interspecific distances are indicated with light grey bars and intraspecific distances with dark grey bars. Left Y-axis: numbers of intraspecific comparisons; Right Y-axis: numbers of interspecific comparisons.

Family	Species	Nearest species	Mean K2P- distance (%)
	Herring Gull Larus argentatus	Yellow-legged Gull L. michahellis	0.14
	Lesser Black-backed Gull Larus fuscus	Caspian Gull L. cachinnans	0
Laridae	Iceland Gull Larus glaucoides	Caspian Gull L. cachinnans	0
	Glaucous Gull Larus hyperboreus	Yellow-legged Gull L. michahellis	0.58
	Yellow-legged Gull Larus michahellis	Species Gull Larus argentatus -backed Gull Larus fuscus Gull Larus glaucoides Gull Larus hyperboreus ed Gull Larus michahellis Caspian Gull L. cachinnans Yellow-legged Gull L. michahellis Caspian Gull L. cachinnans Caspian Gull L. cachinnans	0
Stercorariidae	Great Skua Stercorarius skua	Pomarine Skua S. pomarinus	0.30

Table 2. Bird species (Charadriiformes) from the Netherlands with one or more shared DNA barcodes (K2P-distances of 0%). For a detailed breakdown of the individual samples involved see Appendix – Table 2.

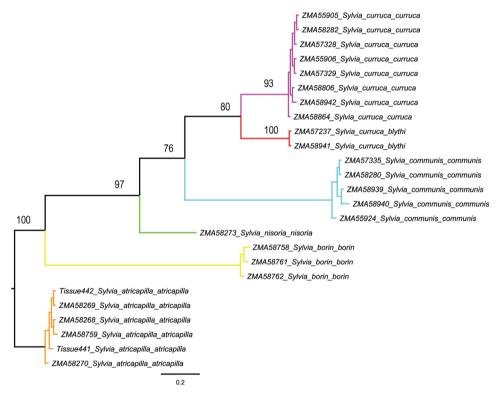


Figure 2. Phylogenetic relationships of two putative subspecies of Lesser Whitethroat, i.e. the Western Lesser Whitethroat *Sylvia curruca curruca* and the Northeastern Lesser Whitethroat *Sylvia curruca blythi* from the Netherlands, based on analysis of 694 bp of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). Bootstrap values are given for the maximum likelihood (ML) analysis.

Although most species possessed low intraspecific distances, one species showed high intraspecific K2P-distances clearly above the threshold of 2 to 3 per cent sequence divergence in our data set. This is the Lesser Whitethroat *Sylvia curruca*, with a mean interspecific divergence of 5.76% and a maximum interspecific distance of 8.68%. Two subspecies occur in the Netherlands, i.e. the Western Lesser Whitethroat *S. c. curruca*

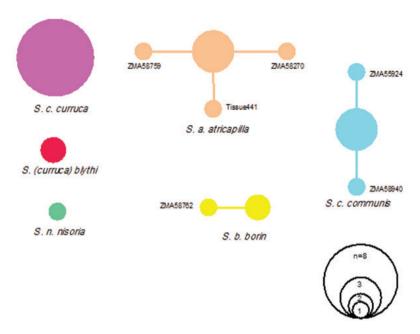


Figure 3. Haplotype networks constructed with statistical parsimony based on 694 bp of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) of the *Sylvia* group (25 individuals). Each circle represents one haplotype; size of circles is proportional to haplotype frequency.

and, as a migrant, the Northeastern Lesser Whitethroat *S. c. blythi*. Both are morphologically somewhat distinct, with compared to the nominate *S. c. blythi* having a paler top of the head, separated from face by a white supercilium, and geographically the nominate occupies the western part of the species range and *S. c. blythi* the eastern part. A maximum likelihood tree for these two taxa based on Kimura 2-parameter is presented in Figure 2. Two different haplotype networks, one each for *S. c. curruca* and *S. c. blythi* were recovered by TCS (Figure 3), and given the large genetic distances between their haplotypes, the two taxa are not included in the same haplotype network.

Discussion

We here present the results of a modest effort to barcode the avifauna of the Netherlands. In terms of DNA barcoding of birds, the Netherlands form the southernmost part of one of the most densely sampled regions globally (Lijtmaer et al. 2012: figure 1). In addition, many of the species that overwinter in the country originate equally well-sampled regions to the north. As such our study adds to a growing number of studies allowing us to build up comprehensive public libraries of bird barcodes. Combined these allow us to explore new lines of scientific inquiry and practical applications (Hebert et al. 2010, Lijtmaer et al. 2012, but see Ebach and Carvalho 2010). The collection of our samples was done as part of the museum's standard collection man-

agement of newly obtained material, and as such sample collection was inexpensive and required little effort in terms of manpower. All birds were collected and processed in the Netherlands and did not require specific permits other than the ones already required to curate the collections.

Recently, Taylor and Harris (2012) expressed the opinion that proponents of DNA barcoding consistently fail to recognize its limitations (including, but not restricted to, the functioning of COI as a universal barcoding gene, whether its use is to be restricted to species identification only or whether it has a role in species discovery and delimitation and the failure to have sufficient systems in place to deal with the large amounts of data generated), do not evolve their methodologies, and do not embrace the possibilities that next-generation sequencing offers. We agree that DNA barcoding will not offer a panacea for all the issues Taylor and Harris (2012) raised, or indeed some of its earlier critics (Will et al. 2005, Moritz and Cicero 2004) but we point out that for this was probably never the intention of DNA barcoding when envisaged some ten years ago. Irrespective of the aims and goals of DNA barcoding as a 'global enterprise' (Ebach and Carvalho 2010), we found it a useful tool in our studies on birds (cf. Baker et al. 2009). The bird collection of the Zoological Museum Amsterdam, and our sample reported in this study, was well-curated by knowledgeable staff, with a very high degree of taxonomic certainty attached to each individual specimen. We see immense value to having a DNA barcoding dataset linked to this reference collection. As such this work has added to the growing library of DNA barcodes of bird species of the world and subsequent improvement in our knowledge of biodiversity.

The mean intraspecific divergences found in the birds of the Netherlands (0.29%, based on 147 species) is congruent with that of for instance Argentina (0.24%, 500 species), North America (0.23%, 643 species) and the Holarctic (0.24%, 566 species) (Kerr et al. 2009a, Aliabadian et al. 2009). More importantly, like other studies on birds, the efficiency of DNA barcode sequences to identify species is high, showing a clear barcoding gap (Figure 1), and overall it seems that for birds typically 95% or more of the species can be identified (Hebert et al. 2003, Johnsen et al. 2010, Kerr et al. 2009a, b, Yoo et al. 2006, Aliabadian et al. 2009).

Most DNA barcoding studies of birds flag a small number of deep divergences (e.g. Johnsen et al. 2010, Kerr et al. 2009b, Aliabadian et al. 2009, Nijman and Aliabadian 2013), in our study involving the two subspecies of *Sylvia curruca*, where the two lineages diverge almost 6%. Similar results were found by Olsson et al. (2013) when analyzing the cytochrome *b* gene for these two taxa, with distances in the order of 11-14%. Based on COI sequences, the two taxa appear to be sister taxa, albeit with a relatively low support (Figure 3), but no other members of the *Sylvia curruca* were included in the analysis. In contrast, having included a range of other members of this complex, Olsson et al. (2013) found *curruca* and *blythi* not to be sister taxa. Olsson et al. (2013: 81) concluded that while "due to their morphological similarity it is unclear where their ranges meet, [o]ur data suggest that *blythi* is a valid taxon, not closely related to *curruca*. It has its closest relatives to the south-east [Asia], and may have colonised the eastern taiga from this direction, ultimately coming into contact

with curruca". When it comes to drawing conclusion from their work with respect to taxomomy, Olsson et al. (2013) were, in our view correctly, cautious. They noted that the Sylvia curruca complex comprised up to 13 taxa with little consensus as to circumscription and taxonomic rank. Of these, morphologically some taxa are very similar, including S. c. curruca and S. c. blythi, and the apparent conflict between morphology and phylogeny (based in their case on cyt b and in our study on COI) can be explained in different ways. One would be to accept the single mitochondrial gene trees at face value in which case the morphological similarities in pelage coloration may be a result of parallel evolution possibly in response to adaptations to similar temperate forest habitats - both taxa are then best treated as different species. Alternatively, the mitochondrial gene trees do not reflect the species tree and, based on morphological similarities, S. c. curruca and S. c. blythi are best treated as sister taxa (either as one or two species). Their divergent position on the mitochondrial gene tree, and the large genetic distances between these taxa, are due to ancient mitochondrial introgression. In either case, working with single mitochondrial markers cannot not resolve this issue and a more integrative approach ideally involving the analysis of nuclear genes is paramount.

Those cases where we found species sharing the same DNA barcodes were small in number but not insignificant. Seven of the eight cases involved closely related gulls with partially overlapping ranges, or allopatric distributions, that are part of a recent Holarctic radiation (Liebers-Helbig et al. 2010). Alternatively, the the sharing of DNA barcodes may be due to hybridization or, perhaps less likely, misidentification. Likewise, skuas are part of a recent radiation with, just like gulls, frequent hybridization between species (Ritz 2009). DNA barcoding using a relative slowly evolving maternally inherited gene, with, compared to other mitochondrial genes, small amounts of rate heterogeneity (Pacheco et al. 2011), will, on its own, not be able to differentiate between these taxa.

We conclude that DNA barcoding approach makes it possible to identify known Dutch bird species with a very high resolution. Although some species were flagged for further detailed taxonomic investigation, our study reaffirms once more that a short segment of COI gene can be used to handle large number of taxa and aid in detecting overlooked taxa and hybridizing species with low deep barcode divergences.

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Appendix

Supplementary Table 1. List of all Dutch birds that have been sequenced in this study, with voucher numbers and collection localities. Note that specimens from which only tissue samples have been taken have not been given a collection number, sine loco refers to specimens collected in the Netherlands but without a precise named collection locality. Localities in the province of Friesland are listed with their Dutch name first, followed by their Frisian name. Coordinates are given in decimal degrees.

0 1 1	ZMA	ъ .	T 10.	Coordi	nates	Access
Species or subspecies	number	Preparation	Locality	N	E	numbers
Accipiter gentilis gentilis	ZMA58297	skin	Zaandam	52.25N,	4.49E	KF946551
Accipiter gentilis gentilis	ZMA58724	skin	De Rips	51.32N,	5.48E	KF946552
Accipiter nisus nisus	ZMA58243	skin	Malden	51.47N,	5.52E	KF946553
Accipiter nisus nisus	ZMA58245	skin	Helden	51.21N,	5.55E	KF946554
Accipiter nisus nisus	ZMA58246	skin	Reuver	51.17N,	6.04E	KF946555
Accipiter nisus nisus	ZMA58247	skin	Culemborg	51.55N,	5.15E	KF946556
Accipiter nisus nisus	ZMA58248	skin	Amsterdam	52.21N,	4.53E	KF946557
Accipiter nisus nisus	ZMA58741	skin	Amsterdam	52.21N,	4.53E	KF946558
Accipiter nisus nisus	ZMA58742	skin	Montfort	51.07N,	5.56E	KF946559
Accipiter nisus nisus	ZMA58743	skin	Belfeld	51.18N,	6.08E	KF946560
Accipiter nisus nisus	ZMA58744	skin	Laren	52.11N,	6.22E	KF946561
Accipiter nisus nisus	ZMA58745	skin	Almere	52.22N,	5.13E	KF946562
Accipiter nisus nisus	ZMA58746	skin	Venlo	51.21N,	6.11E	KF946563
Acrocephalus palustris	ZMA56679	skin	Harderbroek reserve	52.22N,	5.35E	KF946564
Acrocephalus palustris	ZMA58811	skin	Castricum	52.32N,	4.36E	KF946565
Acrocephalus schoenobaenus	ZMA58278	skin	Almere	52.22N,	5.13E	KF946566
Acrocephalus schoenobaenus	ZMA58809	skin	Almere	52.22N,	5.13E	KF946567
Acrocephalus schoenobaenus	ZMA58810	skin	Castricum	52.32N,	4.36E	KF946568
Acrocephalus schoenobaenus	ZMA58862	skin	Wassenaar	53.08N,	5.53E	KF946569
Acrocephalus scirpaceus scirpaceus	ZMA58277	skin	Oostvaardersdijk	52.29N,	5.23E	KF946570
Acrocephalus scirpaceus scirpaceus	ZMA58725	skin	Schermerhorn	52.36N,	4.54E	KF946571
Acrocephalus scirpaceus scirpaceus	ZMA58727	skin	Lelystad	52.29N,	5.24E	KF946572
Acrocephalus scirpaceus scirpaceus	ZMA58728	skin	Lelystad	52.29N,	5.24E	KF946573
Acrocephalus scirpaceus scirpaceus	ZMA58729	skin	Castricum	52.32N,	4.36E	KF946574
Acrocephalus scirpaceus scirpaceus	ZMA58863	skin	Lauwersmeer	53.22N,	6.14E	KF946575

Species on subsection	ZMA	Dronomatic	Logalies	Coordinates	Access
Species or subspecies	number	Preparation	Locality	N E	numbers
Acrocephalus scirpaceus scirpaceus	ZMA58937	skin	Lelystad	52.29N, 5.24E	KF946576
Acrocephalus scirpaceus scirpaceus	ZMA58938	skin	Purmerend	52.28N, 4.58E	KF946577
Aegithalos caudatus europaeus	ZMA57353	skin	Westenschouwen	51.41N, 3.42E	KF946578
Aegithalos caudatus europaeus	ZMA57354	skin	Westenschouwen	51.41N, 3.42E	KF946579
Aegithalos caudatus europaeus	ZMA57356	skin	Hilversum	52.13N, 5.09E	KF946580
Aegithalos caudatus europaeus	ZMA58804	skin	Castricum	52.32N, 4.36E	KF946581
Alcedo atthis ispida	ZMA56216	skin	Haelen	51.13N, 5.56E	KF946582
Alcedo atthis ispida	ZMA57341	skin	Purmerland	52.28N, 4.55E	KF946583
Alcedo atthis ispida	ZMA57342	skin	Alkmaar	52.38N, 4.44E	KF946584
Alcedo atthis ispida	ZMA57343	skin	Utrecht	52.03N, 5.08E	KF946585
Alcedo atthis ispida	ZMA58869	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946586
Alle alle alle	ZMA58842	skin	Amsterdam	52.21N, 4.53E	KF946587
Alle alle alle	ZMA58917	skin	Amsterdam	52.21N, 4.53E	KF946588
Alle alle alle	ZMA58918	skin	Den Helder	52.55N, 4.46E	KF946589
Anas acuta	ZMA58228	skin	Vlieland Island	53.15N, 4.59E	KF946590
Anas strepera strepera	ZMA58913	skin	Driebond Polder	53.11N, 6.37E	KF946591
Anthus spinoletta spinoletta	ZMA58279	skin	Lelystad	52.29N, 5.24E	KF946592
Anthus spinoletta spinoletta	ZMA64552	skin	Castricum	52.32N, 4.36E	KF946593
Anthus trivialis trivialis	Tissue553	DNA sample	Castricum	52.32N, 4.36E	KF946594
Apus apus apus	ZMA58717	skin	Tegelen	51.19N, 6.09E	KF946595
Ardea cinerea cinerea	Tissue434	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946596
Ardea cinerea cinerea	Tissue435	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946597
Asio flammeus flammeus	ZMA58253	skin	Texel Island	53.04N, 4.43E	KF946598
Asio otus otus	Tissue455	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946599
Asio otus otus	ZMA58233	skin	Purmerend	52.28N, 4.58E	KF946600
Asio otus otus	ZMA58234	skin	Zutphen	52.07N, 6.12E	KF946601
Athene noctua vidalii	ZMA58493	skin	Heerhugowaard	52.4N 4.51E	KF946602
Athene noctua vidalii	ZMA58294	skin	Blerick	51.21N, 6.08E	KF946603
Bombycilla garrulus garrulus	ZMA56300	skin	Amsterdam	52.21N, 4.53E	KF946604
Bombycilla garrulus garrulus	ZMA56301	wings	Texel Island	53.04N, 4.43E	KF946605
Bombycilla garrulus garrulus	ZMA58301	wings	Hellendoorn	52.23N, 6.26E	KF946606
Bombycilla japonica	ZMA58302	skin	Amsterdam	52.21N, 4.53E	KF946607
Buteo buteo buteo	Tissue461	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946608
Buteo buteo buteo	ZMA58238	skin	Wieringermeer	52.54N, 5.01E	KF946609
Buteo buteo buteo	ZMA58239	skin	De Rips	51.32N, 5.48E	KF946610
Buteo buteo buteo	ZMA58781	wing	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946611
Buteo buteo buteo	ZMA58828	skin	Wartena	52.12N, 4.3E	KF946612
Buteo buteo buteo	ZMA58920	wings	Rolde	52.58N, 6.38E	KF946613

Cm	ZMA Dunancia		T andte-	Coordinates	Access	
Species or subspecies	number	Preparation	Locality	N E	numbers	
Calidris alpina alpina	ZMA58700	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946614	
Calonectris diomedea borealis	ZMA57255	skin	Lith	51.47N, 5.26E	KF946615	
Carduelis cannabina cannabina	ZMA58911	skin	Noordijk	52.08N, 6.34E	KF946616	
Carduelis carduelis	ZMA58866	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946617	
Carduelis chloris chloris	ZMA57337	skin	Cadier en Keer	50.49N, 5.46E	KF946618	
Carduelis chloris chloris	ZMA58947	skin	Goor	52.14N, 6.34E	KF946619	
Carduelis flammea cabaret	ZMA57248	skin	Kennemerduinen	52.42N, 4.58E	KF946620	
Carduelis flammea cabaret	ZMA58283	skin	Westenschouwen	51.41N, 3.42E	KF946621	
Carduelis flammea flammea	ZMA57251	skin	Kennemerduinen	52.42N, 4.58E	KF946622	
Carduelis flammea flammea	ZMA64564	skin	Castricum	52.32N, 4.36E	KF946623	
Carduelis flavirostris	ZMA57253	skin	Castricum	52.32N, 4.36E	KF946624	
Carduelis flavirostris	ZMA57254	skin	Castricum	52.32N, 4.36E	KF946625	
Carduelis spinus	ZMA55904	skin	Nijverdal	52.22N, 6.28E	KF946626	
Carduelis spinus	ZMA57256	skin	Westenschouwen	51.41N, 3.42E	KF946627	
Carduelis spinus	ZMA58286	skin	Hellendoorn	52.23N, 6.26E	KF946628	
Certhia brachydactyla megarhyncha	ZMA57322	skin	Hellendoorn	52.23N, 6.26E	KF946629	
Certhia brachydactyla megarhyncha	ZMA57323	skin	Lekkerkerk	51.53N, 4.41E	KF946630	
Certhia brachydactyla megarhyncha	ZMA57325	skin	Wageningen	51.58N, 5.38E	KF946631	
Certhia brachydactyla megarhyncha	ZMA57326	skin	Zeist	52.05N, 5.16E	KF946632	
Certhia brachydactyla megarhyncha	ZMA57327	skin	Heiloo	52.36N, 4.44E	KF946633	
Certhia brachydactyla megarhyncha	ZMA58805	skin	Castricum	52.32N, 4.36E	KF946634	
Certhia brachydactyla megarhyncha	ZMA58949	skin	Lekkerkerk	51.53N, 4.41E	KF946635	
Certhia brachydactyla megarhyncha	ZMA64563	skin	Castricum	52.32N, 4.36E	KF946636	
Charadrius hiaticula	Tissue452	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946637	
Circus aeruginosus aeruginosus	ZMA58780	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946638	
Circus aeruginosus aeruginosus	ZMA58826	skin	Eibergen	52.06N, 6.37E	KF946639	
Circus aeruginosus aeruginosus	ZMA58874	wings	Zuid-Flevoland	52.26N, 5.16E	KF946640	
Coccothraustes coccothraustes	ZMA56212	skin	Laag Keppel	51.59N, 6.13E	KF946641	
Corvus corax corax	ZMA57144	skin	Appelscha/ Appelskea	52.55N, 5.2E	KF946642	
Coturnix coturnix coturnix	ZMA58775	skin	Deventer	52.15N, 6.11E	KF946643	
Coturnix coturnix coturnix	ZMA58776	skin	Het Bildt	53.17N, 5.4E	KF946644	
Cuculus canorus canorus	ZMA56681	skin	Bergen	52.4N, 4.41E	KF946645	

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Species or subspecies	number	Preparation	Locality	N E	numbers
Cuculus canorus canorus	ZMA64549	skin	Alkmaar	52.38N, 4.44E	KF946646
Delichon urbicum	ZMA56215	skin	Sea	,	KF946647
Delichon urbicum urbicum	ZMA55919	skin	Nieuwegein	52.01N, 5.05E	KF946648
Delichon urbicum urbicum	ZMA58300	wings	Lage Zwaluwe	51.42N, 4.42E	KF946649
Delichon urbicum urbicum	ZMA58870	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946650
Dendrocopos major pinetorum	ZMA58803	skin	Oudkerk/Aldtsjerk	53.15N, 5.53E	KF946651
Dryocopus martius martius	ZMA58766	skin	Tegelen	51.19N, 6.09E	KF946652
Emberiza citrinella citrinella	ZMA57257	skin	Westenschouwen	51.41N, 3.42E	KF946653
Emberiza melanocephala	ZMA56996	skin	Bovenkerk	52.17N, 4.49E	KF946654
Emberiza pusilla	ZMA58859	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946655
Emberiza pusilla	ZMA58860	skin	Vlieland Island	53.15N, 4.59E	KF946656
Emberiza schoeniclus schoeniclus	ZMA58857	skin	Noordpolderzijl	53.25N, 6.34E	KF946657
Emberiza schoeniclus schoeniclus	ZMA58858	skin	Oostvaardersdijk	52.29N, 5.23E	KF946658
Erithacus rubecula rubecula	Tissue436	DNA sample	Castricum	52.32N, 4.36E	KF946659
Erithacus rubecula rubecula	Tissue437	DNA sample	Castricum	52.32N, 4.36E	KF946660
Erithacus rubecula rubecula	ZMA58274	skin	Bloemendaal	52.24N, 4.33E	KF946661
Erithacus rubecula rubecula	ZMA58740	skin	Doldersum	52.52N, 6.17E	KF946662
Falco columbarius aesalon	ZMA58840	skin	Texel Island	53.04N, 4.43E	KF946663
Falco columbarius aesalon	ZMA60127	skin	Spaarndam	52.24N, 4.41E	KF946664
Falco peregrinus peregrinus	ZMA58872	skin	Haarlem	52.23N, 4.37E	KF946665
Falco subbuteo subbuteo	ZMA56231	skin	Zundert	51.28N, 4.38E	KF946666
Falco subbuteo subbuteo	ZMA56232	skin	Heerhugowaard	52.4N, 4.51E	KF946667
Falco subbuteo subbuteo	ZMA58241	skin	Hoogland	52.1N, 5.21E	KF946668
Falco subbuteo subbuteo	ZMA58242	skin	Texel Island	53.04N, 4.43E	KF946669
Falco subbuteo subbuteo	ZMA58841	skin	Amsterdam	52.21N, 4.53E	KF946670
Falco tinnunculus tinnunculus	Tissue456	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946671
Falco tinnunculus tinnunculus	ZMA58296	skin	Zaandam	52.25N, 4.49E	KF946672
Falco tinnunculus tinnunculus	ZMA58752	skin	Maasbree	51.21N, 6.03E	KF946673
Falco tinnunculus tinnunculus	ZMA58754	skin	Boekend	51.22N, 6.06E	KF946674
Falco tinnunculus tinnunculus	ZMA58774	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946675
Falco tinnunculus tinnunculus	ZMA58837	skin	Westzaan	52.26N, 4.46E	KF946676
Falco tinnunculus tinnunculus	ZMA58838	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946677
Falco tinnunculus tinnunculus	ZMA58839	wings	Reutum	52.23N, 6.5E	KF946678
Falco vespertinus	ZMA58773	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946679
Ficedula hypoleuca muscipeta	ZMA55913	skin	Otterlo	52.04N, 5.5E	KF946680
Ficedula hypoleuca muscipeta	ZMA57239	skin	Markelo	52.14N, 6.3E	KF946681
Ficedula hypoleuca muscipeta	ZMA57320	skin	Garderen	52.12N, 5.43E	KF946682
Ficedula hypoleuca	ZMA58865	skin	Eemshaven	53.26N, 6.52E	KF946683

Canadan an ambarratur	ZMA	D	I 1:	Coordinates	Access	
Species or subspecies	number	Preparation	Locality	N E	numbers	
Fratercula arctica grabae	ZMA56226	skin	Texel Island	53.04N, 4.43E	KF946684	
Fratercula arctica grabae	ZMA58226	skin	Texel Island	53.04N, 4.43E	KF946685	
Fratercula arctica grabae	ZMA58227	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946686	
Fringilla coelebs coelebs	ZMA58948	skin	Goor	52.14N, 6.34E	KF946687	
Fringilla montifringilla	Tissue449	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946688	
Fulmarus glacialis auduboni	ZMA56235	wings	Hondsbossche Zeewering	52.44N, 4.38E	KF946689	
Fulmarus glacialis glacialis	ZMA60119	skin	Neeltje Jans	51.37N, 3.41E	KF946690	
Fulmarus glacialis glacialis	ZMA60120	skin	Texel Island	53.04N, 4.43E	KF946691	
Fulmarus glacialis glacialis	ZMA60121	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946692	
Fulmarus glacialis glacialis	ZMA60123	skin	Ameland Island	53.27N, 5.39E	KF946693	
Fulmarus glacialis glacialis	ZMA60124	skin	Ameland Island	53.27N, 5.39E	KF946694	
Fulmarus glacialis glacialis	ZMA60125	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946695	
Fulmarus glacialis glacialis	ZMA60126	skin	Petten	52.46N, 4.38E	KF946696	
Fulmarus glacialis	ZMA58737	skin	Vlieland Island	53.15N, 4.59E	KF946697	
Gallinula chloropus chloropus	Tissue105	DNA sample	Wijde Wormer	52.28N, 4.53E	KF946698	
Gallinula chloropus chloropus	Tissue110	DNA sample	Wijde Wormer	52.28N, 4.53E	KF946699	
Garrulus glandarius glandarius	ZMA58306	wings	Amsterdam	52.21N, 4.53E	KF946700	
Gavia immer	Tissue214	DNA sample	Bergen aan Zee	52.39N, 4.37E	KF946701	
Haematopus ostralegus ostralegus	Tissue458	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946702	
Haematopus ostralegus ostralegus	Tissue459	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946703	
Hirundo rustica rustica	Tissue450	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946704	
Hirundo rustica rustica	Tissue451	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946705	
Hirundo rustica rustica	ZMA56214	skin	Amstelveen	52.18N, 4.53E	KF946706	
Hirundo rustica rustica	ZMA58289	skin	Appelscha/ Appelskea	52.55N, 5.2E	KF946707	
Hirundo rustica rustica	ZMA58290	skin	Appelscha/ Appelskea	52.55N, 5.2E	KF946708	
Hirundo rustica rustica	ZMA58696	skin	Rijswijk	51.57N, 5.21E	KF946709	
Hirundo rustica rustica	ZMA58802	skin	Noordbergum/ Noardburgum	53.13N, 6E	KF946710	
Jynx torquilla torquilla	ZMA56213	skin	Aarle-Rixtel	51.3N, 5.39E	KF946711	
Jynx torquilla torquilla	ZMA57330	skin	Limmen	52.34N, 4.41E	KF946712	
Jynx torquilla torquilla	ZMA58303	wings	Belfeld	51.18N, 6.08E	KF946713	
Jynx torquilla torquilla	ZMA58873	skin	Wilnis	52.11N, 4.54E	KF946714	
Larus argentatus argenteus	ZMA58921	wings	Eemshaven	53.26N, 6.52E	KF946715	

Consider an author active	ZMA	D	I 1:	Coordinates	Access	
Species or subspecies	number	Preparation	Locality	N E	numbers	
Larus argentatus	Tissue433	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946716	
Larus cachinnans	ZMA64547	skin	Vlieland Island	53.15N, 4.59E	KF946717	
Larus fuscus graelsii	Tissue432	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946718	
Larus fuscus intermedius	Tissue327	DNA- sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946719	
Larus fuscus intermedius	ZMA55932	skin	Neeltje Jans	51.37N, 3.41E	KF946720	
Larus fuscus intermedius	ZMA56230	skin	Europoort	51.56N, 4.05E	KF946721	
Larus fuscus intermedius	ZMA58834	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946722	
Larus glaucoides glaucoides	ZMA58836	wings	Texel Island	53.04N, 4.43E	KF946723	
Larus hyperboreus	ZMA56221	skin	Texel Island	53.04N, 4.43E	KF946724	
Larus melanocephalus	ZMA57226	skin	Wijdenes	52.37N, 5.1E	KF946725	
Larus michahellis michahellis	ZMA58835	skin	Afsluitdijk	52.57N, 5.04E	KF946726	
Limosa lapponica lapponica	ZMA58202	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946727	
Limosa lapponica lapponica	ZMA58203	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946728	
Limosa lapponica taymyrensis	ZMA58204	skin	Paesens	53.24N, 6.06E	KF946729	
Limosa lapponica taymyrensis	ZMA58205	skin	Paesens	53.24N, 6.06E	KF946730	
Limosa lapponica taymyrensis	ZMA58206	skin	Paesens	53.24N, 6.06E	KF946731	
Limosa lapponica taymyrensis	ZMA58207	skin	Paesens	53.24N, 6.06E	KF946732	
Limosa lapponica taymyrensis	ZMA58208	skin	Paesens	53.24N, 6.06E	KF946733	
Limosa lapponica taymyrensis	ZMA58782	wings	Castricum	52.32N, 4.36E	KF946734	
Limosa lapponica taymyrensis	ZMA58783	wings	Castricum	52.32N, 4.36E	KF946735	
Limosa limosa limosa	Tissue457	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946736	
Limosa limosa limosa	ZMA57227	skin	Holysloot	52.24N, 5.01E	KF946737	
Limosa limosa limosa	ZMA58229	skin	Waterland	52.07N, 4.19E	KF946738	
Limosa limosa limosa	ZMA58230	skin	Edam	52.32N, 5.01E	KF946739	
Limosa limosa limosa	ZMA58231	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946740	
Limosa limosa limosa	ZMA58232	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946741	
Locustella luscinioides luscinioides	ZMA64557	skin	Castricum	52.32N, 4.36E	KF946742	
Locustella naevia naevia	ZMA56675	skin	Almere	52.22N, 5.13E	KF946743	
Locustella naevia naevia	ZMA56678	skin	Almere	52.22N, 5.13E	KF946744	
Locustella naevia naevia	ZMA57235	skin	Westenschouwen	51.41N, 3.42E	KF946745	
Locustella naevia naevia	ZMA58812	skin	Castricum	52.32N, 4.36E	KF946746	
Locustella naevia naevia	ZMA58936	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946747	
Locustella naevia naevia	ZMA60132	skin	Kennemerduinen	52.42N, 4.58E	KF946748	
Locustella naevia naevia	ZMA60133	skin	Kennemerduinen	52.42N, 4.58E	KF946749	
Locustella naevia naevia	ZMA64556	skin	Castricum	52.32N, 4.36E	KF946750	

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opecies of subspecies	number	Treparation	Locality	N E	numbers
Loxia curvirostra curvirostra	ZMA57246	skin	Eesveen	52.5N, 6.06E	KF946751
Loxia curvirostra curvirostra	ZMA57247	skin	Leersum	52.01N, 5.25E	KF946752
Luscinia megarhynchos megarhynchos	ZMA58798	skin	Amsterdam	52.21N, 4.53E	KF946753
Lymnocryptes minimus	ZMA55930	skin	Heerhugowaard	52.4N, 4.51E	KF946754
Lymnocryptes minimus	ZMA58293	skin	Uitgeest	52.31N, 4.42E	KF946755
Milvus milvus milvus	ZMA58307	wings	Grolloo	52.55N, 6.39E	KF946756
Milvus milvus milvus	ZMA58824	wings	Susteren	51.03N, 5.52E	KF946757
Milvus milvus milvus	ZMA58825	skin	Heurne	51.54N, 6.34E	KF946758
Motacilla alba yarrellii	ZMA58946	skin	Haastrecht	51.59N, 4.46E	KF946759
Motacilla cinerea cinerea	ZMA57241	skin	Westenschouwen	51.41N, 3.42E	KF946760
Motacilla cinerea cinerea	ZMA58266	skin	Westenschouwen	51.41N, 3.42E	KF946761
Motacilla cinerea cinerea	ZMA58267	skin	Westenschouwen	51.41N, 3.42E	KF946762
Motacilla cinerea cinerea	ZMA58945	skin	Westenschouwen	51.41N, 3.42E	KF946763
Muscicapa striata striata	ZMA57336	skin	Ilpendam	52.27N, 4.56E	KF946764
Numenius arquata arquata	Tissue431	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946765
Numenius arquata arquata	ZMA58765	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946766
Numenius arquata arquata	ZMA58829	skin	Heemskerk	52.3N, 4.36E	KF946767
Oenanthe oenanthe leucorhoa	ZMA58868	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946768
Oenanthe oenanthe oenanthe	ZMA58275	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946769
Oenanthe oenanthe oenanthe	ZMA58800	skin	Noordbergum/ Noardburgum	53.13N, 6E	KF946770
Oriolus oriolus oriolus	ZMA58288	skin	Heteren	51.57N, 5.45E	KF946771
Oriolus oriolus oriolus	ZMA58305	wings	Zundert	51.28N, 4.38E	KF946772
Pandion haliaetus haliaetus	ZMA58823	wing	Vlieland Island	53.15N, 4.59E	KF946773
Panurus biarmicus biarmicus	ZMA57318	skin	Oostvaardersdijk	52.29N, 5.23E	KF946774
Panurus biarmicus biarmicus	ZMA58262	skin	Lelystad	52.29N, 5.24E	KF946775
Panurus biarmicus biarmicus	ZMA58263	skin	Lelystad	52.29N, 5.24E	KF946776
Panurus biarmicus biarmicus	ZMA58264	skin	Lelystad	52.29N, 5.24E	KF946777
Panurus biarmicus biarmicus	ZMA58265	skin	Lelystad	52.29N, 5.24E	KF946778
Panurus biarmicus biarmicus	ZMA58854	skin	Oostvaardersdijk	52.29N, 5.23E	KF946779
Panurus biarmicus biarmicus	ZMA58855	skin	Oostvaardersdijk	52.29N, 5.23E	KF946780
Panurus biarmicus biarmicus	ZMA58856	skin	Oostvaardersdijk	52.29N, 5.23E	KF946781
Parus ater ater	Tissue555	DNA sample	Castricum	52.32N, 4.36E	KF946782
Parus ater ater	ZMA56219	skin	Huizen	52.17N, 5.14E	KF946783
Parus ater ater	ZMA57242	skin	Arnhem	51.58N, 5.53E	KF946784
Parus ater ater	ZMA57243	skin	Amsterdam	52.21N, 4.53E	KF946785
Parus ater ater	ZMA58867	skin	Amsterdam	52.21N, 4.53E	KF946786
Parus ater ater	ZMA64562	skin	Castricum	52.32N, 4.36E	KF946787
Parus caeruleus caeruleus	Tissue438	DNA sample	Castricum	52.32N, 4.36E	KF946788

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Species or subspecies	number	Preparation	Locality	N E	numbers
Parus caeruleus caeruleus	Tissue439	DNA sample	Castricum	52.32N, 4.36E	KF946789
Parus caeruleus caeruleus	Tissue440	DNA sample	Castricum	52.32N, 4.36E	KF946790
Parus caeruleus caeruleus	ZMA58944	wing	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946791
Parus cristatus mitratus	ZMA56677	skin	Nijverdal	52.22N, 6.28E	KF946792
Parus cristatus mitratus	ZMA57245	skin	Hoog Buurlo	52.1N, 5.5E	KF946793
Parus major major	ZMA58796	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946794
Parus major major	ZMA58797	skin	Castricum	52.32N, 4.36E	KF946795
Parus palustris palustris	ZMA57244	skin	Castricum	52.32N, 4.36E	KF946796
Parus palustris palustris	ZMA64561	skin	Goor	52.14N, 6.34E	KF946797
Passer domesticus domesticus	ZMA58799	skin	Cadier en Keer	50.49N, 5.46E	KF946798
Passer domesticus domesticus	ZMA60138	skin	Lekkerkerk	51.53N, 4.41E	KF946799
Passer montanus montanus	ZMA58851	skin	Zuidhorn	53.14N, 6.23E	KF946800
Passer montanus montanus	ZMA58852	skin	Zuidhorn	53.14N, 6.23E	KF946801
Passer montanus montanus	ZMA58853	skin	Zuidhorn	53.14N, 6.23E	KF946802
Passer montanus montanus	ZMA58950	skin	Zuidhorn	53.14N, 6.23E	KF946803
Perdix perdix perdix	ZMA58738	skin	Texel Island	53.04N, 4.43E	KF946804
Perdix perdix perdix	ZMA58739	skin	Petten	52.46N, 4.38E	KF946805
Pernis apivorus	ZMA58827	wings	Vledder	52.53N, 6.13E	KF946806
Phalacrocorax aristotelis aristotelis	ZMA58224	skin	Wijk aan Zee	52.28N, 4.34E	KF946807
Philomachus pugnax	ZMA56680	skin	Graftermeer polder	52.33N, 4.48E	KF946808
Philomachus pugnax	ZMA58250	skin	Lelystad	52.29N, 5.24E	KF946809
Phoenicopterus chilensis	ZMA56683	skin	Ransdorp	52.23N, 4.59E	KF946810
Phoenicurus phoenicurus phoenicurus	ZMA55914	skin	Westenschouwen	51.41N, 3.42E	KF946811
Phylloscopus collybita collybita	ZMA55917	skin	Nijverdal	52.22N, 6.28E	KF946812
Phylloscopus collybita collybita	ZMA55918	wings	Leveroy	51.14N, 5.5E	KF946813
Phylloscopus collybita collybita	ZMA56217	skin	Hoogland	52.1N, 5.21E	KF946814
Phylloscopus trochilus	ZMA58284	skin	Lelystad	52.29N, 5.24E	KF946815
Phylloscopus trochilus	ZMA58710	skin	Almere	52.22N, 5.13E	KF946816
Phylloscopus trochilus	ZMA58713	skin	Egmond aan Zee	52.37N, 4.38E	KF946817
Phylloscopus trochilus	ZMA58714	skin	Lekkerkerk	51.53N, 4.41E	KF946818
Phylloscopus trochilus	ZMA58715	skin	Texel Island	53.04N, 4.43E	KF946819
Phylloscopus trochilus	ZMA58716	skin	Castricum	52.32N, 4.36E	KF946820
Phylloscopus trochilus	ZMA58861	skin	Castricum	52.32N, 4.36E	KF946821
Phylloscopus trochilus	ZMA58933	wings	Goor	52.14N, 6.34E	KF946822
Phylloscopus trochilus	ZMA58934	skin	Eemshaven	53.26N, 6.52E	KF946823
Picus viridis viridis	ZMA58718	skin	Breda	51.33N, 4.46E	KF946824
Picus viridis viridis	ZMA58719	skin	Haaksbergen	52.08N, 6.4E	KF946825
Picus viridis viridis	ZMA58720	skin	Alkmaar	52.38N, 4.44E	KF946826
Picus viridis viridis	ZMA58721	skin	Roggel	51.17N, 5.54E	KF946827
Picus viridis viridis	ZMA58722	skin	Bergen	52.4N, 4.41E	KF946828
Plectrophenax nivalis insulae	ZMA56672	skin	Castricum	52.32N, 4.36E	KF946829

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Pluvialis apricaria	ZMA58213	skin	Winsum	53.09N, 5.38E	KF946830
Pluvialis apricaria	ZMA58214	skin	Winsum	53.09N, 5.38E	KF946831
Pluvialis apricaria	ZMA58215	skin	Dronrijp/Dronryp	53.11N, 5.4E	KF946832
Pluvialis squatarola squatarola	ZMA56224	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946833
Pluvialis squatarola squatarola	ZMA56225	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946834
Puffinus gravis	ZMA64542	skin	Sexbierum/ Seisbierrum	53.14N, 5.28E	KF946835
Pyrrhula pyrrhula europoea	ZMA56673	skin	Castricum	52.32N, 4.36E	KF946836
Pyrrhula pyrrhula europoea	ZMA58793	skin	Castricum	52.32N, 4.36E	KF946837
Pyrrhula pyrrhula europoea	ZMA58794	skin	Castricum	52.32N, 4.36E	KF946838
Pyrrhula pyrrhula europoea	ZMA58795	skin	Castricum	52.32N, 4.36E	KF946839
Pyrrhula pyrrhula europoea	ZMA60137	wings	Kennemerduinen	52.42N, 4.58E	KF946840
Rallus aquaticus aquaticus	ZMA58763	skin	Lauwersmeer	53.22N, 6.14E	KF946841
Recurvirostra avosetta	ZMA58216	skin	Petten	52.46N, 4.38E	KF946842
Regulus ignicapilla ignicapilla	Tissue448	DNA sample	Castricum	52.32N, 4.36E	KF946843
Regulus ignicapilla ignicapilla	ZMA57360	skin	Zundert	51.28N, 4.38E	KF946844
Regulus ignicapilla ignicapilla	ZMA58807	skin	Castricum	52.32N, 4.36E	KF946845
Regulus ignicapilla ignicapilla	ZMA58808	skin	Castricum	52.32N, 4.36E	KF946846
Regulus regulus regulus	ZMA64560	skin	Castricum	52.32N, 4.36E	KF946847
Riparia riparia riparia	ZMA58871	skin	Zeewolde	52.21N, 5.34E	KF946848
Saxicola rubetra	ZMA60131	skin	Kennemerduinen	52.42N, 4.58E	KF946849
Saxicola rubetra	ZMA64555	skin	Castricum	52.32N, 4.36E	KF946850
Somateria mollissima mollissima	ZMA58912	skin	Lauwersoog	53.24N, 6.12E	KF946851
Stercorarius longicaudus	ZMA58779	wings	Afsluitdijk	52.57N, 5.04E	KF946852
Stercorarius longicaudus	ZMA64546	skin	Petten	52.46N, 4.38E	KF946853
Stercorarius parasiticus	ZMA56229	skin	Vlieland Island	53.15N, 4.59E	KF946854
Stercorarius parasiticus	ZMA56684	wings	Terschelling Island	53.26N, 5.29E	KF946855
Stercorarius parasiticus	ZMA58778	skin	Den Oever	52.56N, 5.02E	KF946856
Stercorarius parasiticus	ZMA58830	skin	Den Helder	52.55N, 4.46E	KF946857
Stercorarius pomarinus	Tissue211	DNA sample	Texel Island	53.04N, 4.43E	KF946858
Stercorarius pomarinus	ZMA55929	skin	Hondsbossche Zeewering	52.44N, 4.38E	KF946859
Stercorarius skua skua	ZMA64545	skin	Egmond aan Zee	52.37N, 4.38E	KF946860
Sterna albifrons albifrons	ZMA58832	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946861
Sterna hirundo hirundo	ZMA58915	skin	Eemshaven	53.26N, 6.52E	KF946862
Sterna paradisaea	ZMA58831	skin	Amsterdam	52.21N, 4.53E	KF946863
Streptopelia decaocto decaocto	ZMA58923	wing	Hoogkerk	53.12N, 6.3E	KF946864
Streptopelia turtur turtur	ZMA58757	skin	Texel Island	53.04N, 4.43E	KF946865
Sylvia atricapilla atricapilla	Tissue441	DNA sample	Castricum	52.32N, 4.36E	KF946866
Sylvia atricapilla atricapilla	Tissue442	DNA sample	Castricum	52.32N, 4.36E	KF946867
Sylvia atricapilla atricapilla	ZMA58268	skin	Bloemendaal	52.24N, 4.33E	KF946868

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Sylvia atricapilla atricapilla	ZMA58269	skin	Bloemendaal	52.24N, 4.33E	KF946869
Sylvia atricapilla atricapilla	ZMA58270	skin	Bloemendaal	52.24N, 4.33E	KF946870
Sylvia atricapilla atricapilla	ZMA58759	skin	Cadier en Keer	50.49N, 5.46E	KF946871
Sylvia borin borin	Tissue443	DNA sample	Castricum	52.32N, 4.36E	KF946872
Sylvia borin borin	ZMA58758	skin	Groningen	53.14N, 6.35E	KF946873
Sylvia borin borin	ZMA58761	skin	Almere	52.22N, 5.13E	KF946874
Sylvia borin borin	ZMA58762	skin	Purmerend	52.28N, 4.58E	KF946875
Sylvia communis communis	ZMA55924	wing	Asten	51.21N, 5.48E	KF946876
Sylvia communis communis	ZMA57335	skin	Almere	52.22N, 5.13E	KF946877
Sylvia communis communis	ZMA58280	skin	Breda	51.33N, 4.46E	KF946878
Sylvia communis communis	ZMA58939	skin	Castricum	52.32N, 4.36E	KF946879
Sylvia communis communis	ZMA58940	skin	Bloemendaal	52.24N, 4.33E	KF946880
Sylvia curruca blythi	ZMA58941	skin	Houten	52.01N, 5.1E	KF946881
Sylvia curruca blythi	ZMA57237	skin	Rotterdam	51.57N, 4.32E	KF946882
Sylvia curruca curruca	ZMA55905	skin	Westenschouwen	51.41N, 3.42E	KF946883
Sylvia curruca curruca	ZMA55906	skin	Amsterdam	52.21N, 4.53E	KF946884
Sylvia curruca curruca	ZMA57328	skin	Almere	52.22N, 5.13E	KF946885
Sylvia curruca curruca	ZMA57329	skin	Texel Island	53.04N, 4.43E	KF946886
Sylvia curruca curruca	ZMA58282	skin	Zeewolde	52.21N, 5.34E	KF946887
Sylvia curruca curruca	ZMA58806	skin	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946888
Sylvia curruca curruca	ZMA58864	skin	Eemshaven	53.26N, 6.52E	KF946889
Sylvia curruca curruca	ZMA58942	skin	Bloemendaal	52.24N, 4.33E	KF946890
Sylvia nisoria nisoria	ZMA58273	skin	Westenschouwen	51.41N, 3.42E	KF946891
Tringa ochropus	ZMA64544	skin	Castricum	52.32N, 4.36E	KF946892
Tringa totanus totanus	ZMA58212	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946893
Troglodytes troglodytes troglodytes	Tissue447	DNA sample	Castricum	52.32N, 4.36E	KF946894
Troglodytes troglodytes troglodytes	ZMA58281	skin	Bloemendaal	52.24N, 4.33E	KF946895
Turdus iliacus iliacus	ZMA58287	skin	Bloemendaal	52.24N, 4.33E	KF946896
Turdus merula merula	ZMA56669	skin	Haarlem	52.23N, 4.37E	KF946897
Turdus merula merula	ZMA56670	skin	Bergen	52.4N, 4.41E	KF946898
Turdus merula merula	ZMA57345	skin	Zwolle	52.3N, 6.06E	KF946899
Turdus merula merula	ZMA58731	skin	Alkmaar	52.38N, 4.44E	KF946900
Turdus merula merula	ZMA58732	skin	Maasbree	51.21N, 6.03E	KF946901
Turdus merula merula	ZMA58733	skin	Maasbree	51.21N, 6.03E	KF946902
Turdus merula merula	ZMA58734	skin	Steijl	51.2N, 6.07E	KF946903
Turdus merula merula	ZMA58736	skin	Schiermonnikoog Island	53.29N, 6.11E	KF946904
Turdus philomelos philomelos	Tissue453	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946905
Turdus philomelos philomelos	Tissue454	DNA sample	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946906
Turdus torquatus torquatus	ZMA56222	skin	Texel Island	53.04N, 4.43E	KF946907

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Turdus torquatus torquatus	ZMA56671	skin	Castricum	52.32N, 4.36E	KF946908
Turdus torquatus torquatus	ZMA58693	skin	Apeldoorn	52.1N, 5.58E	KF946909
Turdus torquatus torquatus	ZMA58694	skin	Vlieland Island	53.15N, 4.59E	KF946910
Turdus torquatus torquatus	ZMA58695	skin	Zuilichem	51.48N, 5.07E	KF946911
Turdus torquatus torquatus	ZMA64554	skin	Texel Island	53.04N, 4.43E	KF946912
Turdus viscivorus viscivorus	ZMA60130	skin	Kennemerduinen	52.42N, 4.58E	KF946913
Tyto alba alba	ZMA56233	skin	Burgerbrug	52.45N, 4.42E	KF946914
Tyto alba guttata	ZMA56682	skin	Wierden	52.22N, 6.34E	KF946915
Tyto alba guttata	ZMA58235	skin	Texel Island	53.04N, 4.43E	KF946916
Tyto alba guttata	ZMA58236	skin	Ouderkerk aan de Amstel	52.17N, 4.56E	KF946917
Tyto alba guttata	ZMA58843	skin	Westzaan	52.26N, 4.46E	KF946918
Tyto alba guttata	ZMA58844	skin	Zaanstreek	52.28N, 4.44E	KF946919
Tyto alba guttata	ZMA58845	skin	Roodkerk/ Readtsjerk	53.15N, 5.55E	KF946920
Tyto alba guttata	ZMA58846	skin	Garijp/Garyp	53.1N, 5.57E	KF946921
Tyto alba guttata	ZMA58847	skin	Middenmeer	52.48N, 4.59E	KF946922
Tyto alba guttata	ZMA58848	wings	Leeuwarden/ Ljouwert	53.13N, 5.45E	KF946923
Tyto alba guttata	ZMA58919	skin	Texel Island	53.04N, 4.43E	KF946924
Tyto alba guttata	ZMA64550	skin	Purmerend	52.28N, 4.58E	KF946925
Tyto alba guttata	ZMA64551	skin	Goor	52.14N, 6.34E	KF946926
Uria aalge albionis	ZMA56227	skin	Amsterdam	52.21N, 4.53E	KF946927
Uria aalge albionis	ZMA58218	skin	Vlieland Island	53.15N, 4.59E	KF946928
Uria aalge albionis	ZMA58916	skin	Petten	52.46N, 4.38E	KF946929
Vanellus vanellus	ZMA58784	wing	Valkenburg	52.09N, 4.25E	KF946930
Vanellus vanellus	ZMA58785	wing	Valkenburg	52.09N, 4.25E	KF946931
Vanellus vanellus	ZMA58786	wing	Valkenburg	52.09N, 4.25E	KF946932
Vanellus vanellus	ZMA58787	wing	Valkenburg	52.09N, 4.25E	KF946933
Vanellus vanellus	ZMA58788	wing	Valkenburg	52.09N, 4.25E	KF946934
Vanellus vanellus	ZMA58789	wing	Valkenburg	52.09N, 4.25E	KF946935
Vanellus vanellus	ZMA58790	wing	Valkenburg	52.09N, 4.25E	KF946936
Vanellus vanellus	ZMA58791	wing	Valkenburg	52.09N, 4.25E	KF946937

Supplementary Table 2. Bird species (gulls *Larus* and skuas *Stercorarius*) from the Netherlands with low (< 1.1%) K2P mean intraspecific distances.

Collection number and species		Collection nur	Collection number and species		
#ZMA58835	Larus michahellis	#Tissue327	L. fuscus	0	
#ZMA58835	Larus michahellis	#Tissue432	L. fuscus	0	
#ZMA58835	Larus michahellis	#ZMA55932	L. fuscus	0	
#ZMA58835	Larus michahellis	#ZMA56230	L. fuscus	0	
#ZMA64547	Larus cachinnans	#Tissue327	L. fuscus	0	
#ZMA64547	Larus cachinnans	#Tissue432	L. fuscus	0	
#ZMA64547	Larus cachinnans	#ZMA55932	L. fuscus	0	
#ZMA64547	Larus cachinnans	#ZMA56230	L. fuscus	0	
#ZMA64547	Larus cachinnans	#ZMA58835	L. michahellis	0	
#ZMA58921	Larus argentatus	#ZMA55932	L. fuscus	0.14	
#ZMA58921	Larus argentatus	#ZMA58835	L. michahellis	0.14	
#ZMA58921	Larus argentatus	#Tissue432	L. fuscus	0.15	
#ZMA58921	Larus argentatus	#ZMA56230	L. fuscus	0.15	
#ZMA64547	Larus cachinnans	#ZMA58834	L. fuscus	0.15	
#ZMA64547	Larus cachinnans	#ZMA58921	L. argentatus	0.15	
#ZMA58921	Larus argentatus	#Tissue327	L. fuscus	0.16	
#ZMA55932	Larus fuscus	#Tissue433	L. argentatus	0.29	
#ZMA58835	Larus michahellis	#Tissue433	L. argentatus	0.29	
#Tissue433	Larus argentatus	#Tissue432	L. fuscus	0.30	
#ZMA56230	Larus fusca	#Tissue433	L. argentatus	0.30	
#ZMA64545	Stercorarius skua	#ZMA55929	S. pomarinus	0.30	
#ZMA58836	Larus glaucoides	#Tissue432	L. fuscus	0.31	
#ZMA58836	Larus glaucoides	#ZMA55932	L. fuscus	0.31	
#ZMA58836	Larus glaucoides	#ZMA56230	L. fuscus	0.31	
#ZMA58836	Larus glaucoides	#ZMA58835	L. michahellis	0.31	
#ZMA64547	Larus cachinnans	#Tissue433	L. argentatus	0.31	
#ZMA64547	Larus cachinnans	#ZMA58836	L. glaucoides	0.31	
#Tissue433	Larus argentatus	#Tissue327	L. fuscus	0.32	
#ZMA58836	Larus glaucoides	#Tissue327	L. fuscus	0.32	
#ZMA64545	Stercorarius skua	#Tissue211	S. pomarinus	0.43	
#ZMA58835	Larus michahellis	#ZMA58834	L. fuscus	0.45	
#ZMA58836	Larus glaucoides	#ZMA58834	L. fuscus	0.46	
#ZMA58921	Larus argentatus	#ZMA58836	L. glaucoides	0.46	
#ZMA56221	Larus hyperboreus	#ZMA55932	L. fuscus	0.58	
#ZMA58835	Larus michahellis	#ZMA56221	L. hyperboreus	0.58	
#ZMA56221	Larus hyperboreus	#Tissue432	L. fuscus	0.60	
#ZMA56230	Larus fuscus	#ZMA56221	L. hyperboreus	0.60	
#ZMA58921	Larus argentatus	#ZMA58834	L. fuscus	0.60	
#ZMA64547	Larus cachinnans	#ZMA56221	L. hyperboreus	0.61	
#ZMA58836	Larus glaucoides	#Tissue433	L. argentatus	0.62	
#ZMA56221	Larus hyperboreus	#Tissue327	L. fuscus	0.64	
#ZMA58921	Larus argentatus	#ZMA56221	L. hyperboreus	0.73	
#ZMA58834	Larus fuscus	#Tissue433	L. argentatus	0.75	
#ZMA56221	Larus hyperboreus	#Tissue433	L. argentatus	0.87	
#ZMA58836	Larus glaucoides	#ZMA56221	L. hyperboreus	0.93	
#ZMA58834	Larus fuscus	#ZMA56221	L. hyperboreus	1.06	